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Application of a nonlinear optimization tool to balance diets with constant metabolizability

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ABSTRACT

The goals of this study were to evaluate the ability of a nonlinear optimization tool to provide solutions for maintaining consistent qualities of diets irrespective of the plane of nutrition (L) and to evaluate the effect of the plane of nutrition on intake and digestibility of dry matter (DM), organic matter (OM), crude protein (CP), crude fat (CF), non fibrous carbohydrates (NFC), neutral detergent fiber (NDF), and metabolizability (q_m) of diets using sheep as a generalized experimental model. Eight wethers were randomly assigned to two balanced four-treatment Latin squares conducted simultaneously with four diets providing nutritional levels that were multiples of maintenance levels ($ME = M_m$; $1.5M_m$; $2M_m$; and $2.5M_m$, where ME is the metabolizable energy intake, and M_m is the metabolizable energy intake for maintenance). The ME, M_m, metabolizable protein (MP) and NDF of the diet were subjected to nonlinear constraints; the model was considered a general nonlinear programming problem and solved using Microsoft Excel Solver® with Newton's method of resolution. The intake of nutrients, digestible nutrients, digestible energy (DE) and the amounts of feces and urine produced daily were measured and analyzed statistically by fitting a linear mixed model. The corrected metabolizability (q'_m) and plane of nutrition (L_c) were obtained on the basis of the digestible, urinary, and simulated methane losses. The trends of some variables were reanalyzed by regressing observed values against L_c. All measured variables were affected by L. The intakes of DM and OM increased in an asymptotic fashion as L_c increased, whereas the intake of NDF increased linearly as L_c increased. At levels immediately below maintenance, observed values were approximately constant. Digestible amounts of OM, DE, CF, and CP consumed increased linearly at levels above maintenance, whereas the digestible amounts of total carbohydrates, neutral detergent solubles, and ashes increased in an asymptotic fashion. Under conditions of controlled feeding, the nonlinear optimization tool yielded dietary solutions with a nearly constant metabolizability in which the rate of increase in crude protein, digestible crude protein, and digestible energy intakes remained constant as the plane of nutrition increased.

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1. Introduction

Minimum cost formulations based on linear programming have been used as practical tools for feeding farm ruminants (AFRC, 1993). However, the modern ruminant dietary models are essentially nonlinear for many variables (Hertzler, 1988). Because of the work of Lasdon et al. (1978) who presented a generalized reduced gradient code for nonlinear programming, and as computer hardware and software powers increase and personal computers and commercial spreadsheets are disseminated worldwide, it has become possible to program the nonlinear optimization of diets based on current feeding systems on an ad hoc basis. Complex models relating nutrient requirements and feed nutritive values have been professionally programmed using nonlinear optimization and are currently available for specific farm ruminant species (Tedeschi et al., 2008). Nonetheless, professionally programmed software offers few or no opportunity to the user for modifying the algorithm of resolution, whereas in ad hoc programmed spreadsheet the user can exploit the benefit of pooling information from different models. There is no doubt that the spreadsheet solution is a more tedious programming tool than professionally programmed software, but spreadsheets are widely available and the building of the constraints is a simple and logical programming task.

In spite of the progress made in the quantitative description of diets for ruminants by means of nutritional models, there are systematic errors in these models related to the digestibility and the nutritive value of diets; therefore, further refinements of these models are needed (Joyce et al., 1975; Offner and Sauvant, 2004; Vieira et al., 2008). The constraints related to fiber intake capacity and the required minimum amount of fiber must be incorporated into current feeding systems along with energy and protein constraints (Vieira et al., 2008; Henrique et al., 2011). Obviously, studies must include measurements to evaluate performance predictions (Henrique et al., 2005; 2011; Joyce et al., 1975; Tedeschi et al., 2008; 2012), but little information can be drawn from such studies on systematic errors related to the digestibility of nutrients. The observed data gathered under controlled feeding experiments may be useful in detecting anomalies in trends describing intake and digestibility. Therefore, describing the trends in nutrient intake and digestibility is the first step in evaluating whether these variables behave according to the underlying theories upon which models such as the AFRC (1993) were built. In this context, our goal was to evaluate the trends in intake and digestibility of major nutrients in sheep fed minimum-cost diets formulated at multiple maintenance levels with constant metabolizability by means of a nonlinear optimization tool for personal computers.

2. Material and methods

Replicated balanced four-treatments Latin squares (Lucas, 1957) were conducted simultaneously from April 12th to July 8th of 2011. Treatments consisted of four planes of nutrition (L) that provided nutrition levels that were multiples of maintenance levels, i.e, $L=ME/M_m$ as defined in the AFRC (1993). The metabolizable energy supplied by the diet and the metabolizable energy required for maintenance corresponded to ME and M_m (MJ/d), respectively. The four levels were planned as follows: $ME=M_m$; $ME=1.5M_m$; $ME=2M_m$; and $ME=2.5M_m$.

2.1. Animals, feeding, and duration of the experiment

Eight F1 Dorper × Santa Inês wethers weighing approximately 38.7 kg (standard deviation = 2.8 kg) at the beginning of the experiment were randomly assigned to columns in the Latin squares. Animals were dewormed and kept individually in metabolism cages with free access to water and fed the experimental diets accordingly during the course of the experiment.

Wethers were harnessed with fecal collection bags throughout the four periods of the simultaneous Latin squares. The periods lasted 22 d and were divided into an adaptation period of 15 d and a collection period of seven days. Feed offered and refused (orts), feces, and urine were collected on a daily basis during the collection period. Animals were fed twice at 8:00am and 4:00pm, and orts were collected before each meal. Feces were collected at 9:00am during the collection period. The weights of the animals were recorded on the first and 22nd days, and an average weight was computed for each treatment × animal × period combination. After collection, daily samples were composited accordingly. Urine was allowed to drain into 5000 mL plastic pots containing 50 mL of 1.2 M HCl, and collected, weighed, and sampled twice a day (morning and evening). The fresh weights of the offered and refused diets, feces, and urine were recorded to the nearest 0.005 kg.

2.2. Formulating the experimental diets

The maintenance requirements of the sheep were computed according to AFRC (1993). The problem of formulating different diets that could provide nutritional levels that were multiples of maintenance levels was addressed by treating the model as a general nonlinear programming problem. The problem was programmed using Microsoft Excel Solver® with Newton's method of resolution (Lasdon et al., 1978). The objective function is shown in Eq. (1), which contains the individual costs (c_j, \$/kg) of an unknown quantity x_j of the j-th feedstuff (as fed, kg/d). The constraints are shown in Eqs. (2)–(10).

$$\min \sum_j c_j x_j, \text{ subjected to} \tag{1}$$

$$L = 1,\ 1.5,\ 2, \quad \text{or} \quad 2.5 \tag{2}$$

$$\Delta W \geq 0 \tag{3}$$

$$q_m = 0.55 \tag{4}$$

$$0 \leq \text{Urea} \leq 0.4\ \text{g}/(\text{d} \times \text{kg}) \tag{5}$$

$$ME = LM_m \tag{6}$$

$$MP = 6.5LM_m \tag{7}$$

$$[peNDF] \geq 200 \tag{8}$$

$$F_{NDF} \le 12W \tag{9}$$

$$x_j \ge 0 \tag{10}$$

The plane of nutrition was planned (L) as shown in Eq. (2) and a solution was obtained for each planned value of L. The daily liveweight gain (ΔW, g/d) was set to vary loosely. The metabolizability of the diet ($q_m = [ME]/18.8$, dimensionless) was held constant for all planned values of L (Eq. (4)). The metabolizable energy of the diet (MJ/kg) was computed as follows: $[ME] = (\sum_j [DM]_j[ME]_j x_j)/(\sum_j [DM]_j x_j)$. The amount of offered dry matter (ODM, kg/d) was computed as $\sum_j 0.001[DM]_j x_j$. The metabolizable energy provided by the offered diet (ME, MJ/d) was equal to $\sum_j 0.001[DM]_j[ME]_j x_j$. The dry matter ($[DM]_j$, g/kg as fed), the metabolizable energy ($[ME]_j$, MJ/kg DM) contents, other chemical constituents of the j-th feedstuffs used as inputs to the problem, and the daily amounts x_j offered are shown in Table 1. The tabular metabolizable energy values were used because they are readily available in the AFRC publication. By constraining diets to a constant metabolizable protein (MP, g/d) to ME ratio (g/MJ), (namely $MP:ME$) and by fixing exact values for L (Eq. (2)), dietary ME and MP became functions of M_m as shown in Eqs. (6) and (7). The constants 0.55 (Eq. (4)) and 6.5 (Eq. (7)) were the lowest values used as inputs that resulted in viable solutions for the nonlinear problem in the planned L range. The amount of urea (Urea, g/d) was constrained to an upper limit to avoid ammonia intoxication (Eq. (5)). The fiber-related constraints described in Eqs. (8)–(9) were added to the original AFRC equations. The fibrous content of the j-th feedstuff ($[NDF]_j$, g/kg DM) and its physically effective fiber factor (pef_j, dimensionless) are shown in Table 1. The content of physically effective fiber of the diet ($[peNDF]$, g/kg DM) was set to a minimum required amount as shown in Eq. (8) to avoid rumen dysfunction (Cannas et al., 2003). In addition, the empirical maximum fiber intake capacity (Eq. (9)) was set to 12 g/(d × kg) (Mertens, 1987; Vieira et al., 2008). The amount of fiber offered ($Offered_{NDF}$, g/d) was defined as $10^{-6}\sum_j [DM]_j[NDF]_j x_j$.

Table 1
Costs and chemical composition[a] of the feedstuffs used as inputs to optimize the treatment diets, and the resulting optimized diets for each plane of nutrition.

Inputs[a]	Ingredients			
	Corn Silage	Grounded Corn	Urea	Soybean meal
c[ab], R$/kg as fed	0.12	0.60	1.20	0.60
$[DM]$[a], g/kg as fed	360	860	950	890
$[NDF]$[a] g/kg DM	500	90	–	140
pef [a,f]	0.9	0.34	–	0.23
$[ME]$[a], MJ/kg	9.0	13.8	–	12.6
$[FME]$[a], MJ/kg	7.0	12.4	–	12.0
$[CP]$[a], g/kg DM	70	102	2600	497
$[ADIN]$[a], g/kg DM	1.2	–	–	2.2
a[a], dmls[c]	0.66	0.26	1.0	0.08
b[a], dmls[c]	0.19	0.69	–	0.92
k_d[a], 1/h	0.20	0.01	–	0.08
u[ac], dmls	0.15	0.05	–	–
$[QDP]$[a], g/kg DM	46.2	26.5	2600	39.8
$[SDP]$[a], g/kg DM	12.1	23.9	–	367.8
$[ERDP]$[a], g/kg DM	49.1	45.1	2080	399.6
$[UDP]$[a], g/kg DM	11.7	51.6	–	89.4
$[DUP]$[a], g/kg DM	3.8	46.4	–	68.1
Optimized diets [d]				
1 × maintenance	0.849	0.112	0.002	0.048
1.5 × maintenance	1.289	0.172	–	0.055
2 × maintenance	1.717	0.231	–	0.070
2.5 × maintenance	2.080	0.321	0.006	0.053

[a] c, feed cost; DM, dry matter; NDF, neutral detergent fiber; pef, physically effective fiber; a, instantly degradable soluble fraction; b, insoluble potentially degradable fraction; k_d, fractional degradation rate of b; u, unavailable protein fraction; ME, metabolizable energy; FEM, fermentable metabolizable energy; CP, crude protein; $ADIN$, acid detergent insoluble nitrogen; QPD, quickly degradable protein; UDP, undegradable protein; SDP, slowly degradable protein; $ERDP$, effective rumen degradable protein.

[b] Monetary units per kg of the feedstuff as fed. Prices taken on March, 2011, when R$ 1,00 (Brazilian currency) = US$ 0.60.

[c] Dimensionless.

[d] Amounts of ingredients as fed, i.e., g/d.

2.3. Chemical analyses of the offered diets, orts, feces, and urine

Samples of the offered diets, orts, and feces were dried at 55 °C for 72 h in a forced air oven. Individual samples of the offered diets, orts, and feces were composited on the basis of the air-dried-weight. The composite samples were ground through a 5 mm screen in a Wiley-type mill and stored. Samples of approximately 0.1 kg of the stored samples were ground through a 1 mm screen for chemical analyses. Urine samples were composited by taking 20% of the amount of urine produced daily during the experimental period. The composite urine samples were freeze-dried.

Samples of diets, orts, and feces were analyzed for dry matter ([DM], g/kg as fed; Undersander et al., 1993), crude fat ([CF], g/kg DM; method 2003.06; Thiex et al., 2003) and ash ($[Ash]$, g/kg DM; method 942.05; AOAC, 1998). The nitrogen content of the freeze-dried urine ($[UN]$, g/kg DM) and the crude protein content ($[CP]$, g/kg DM) of the offered diets, orts, and feces were obtained by digesting approx. 0.25 g samples in 100 mL tubes to which 5 mL of H_2SO_4 and 1 g of a 56:1 (w/w) mixture of Na_2SO_4 and $Cu_2SO_4 \cdot 5H_2O$ were added; tubes were then heated in aluminum digestion blocks. This method was performed according to the guidelines outlined in methods 984.13 and 2001.11, including N recovery with certified $NH_4H_2PO_4$ and lysine-HCl (AOAC, 1998; Thiex et al., 2002). The insoluble fiber content ($[NDF]$, g/kg DM) was assayed with sodium sulfite and two additions of a standardized solution of heat-stable amylase, and with ashes excluded according to method 2002.04 (Mertens, 2002). The neutral detergent solubles ($[NDS]$, g/kg DM) and non-fibrous carbohydrates ($[NFC]$, g/kg DM) were estimated as the differences $[NDS]5f = 1000-[NDF]$, and $[NFC] = 1000-[CP]-[CF]-[Ash]-[NDF]$, respectively.

2.4. Computing amounts and contents

The apparent digestible energy contents ([DE], MJ/kg) of the consumed diets were estimated by accounting for the heats of combustion of protein (23.4 MJ/kg), carbohydrates (17.6 MJ/kg), and fat (39.3 MJ/kg) according to Maynard et al. (1979), as follows:

$$DE\ (\mathrm{MJ/d}) = 17.6(D_{NFC}+D_{NDF})+23.4D_{CP}+39.3D_{CF} \quad (11)$$

$$\forall D_{Nut}\,(\mathrm{kg/d}) = Offered_{Nut}-O_{Nut}-R_{Nut} \quad (12)$$

$$[DE]\ (\mathrm{MJ/kg}) = DE/F \quad (13)$$

The actual dry matter intake (F, kg/d) of the diets was computed as $F=0.001(\sum_j [DM]_j x_j-[DM]_O O)$. The amount of daily orts (O, kg/d), the DM content of the orts ($[DM]_O$, g/kg DM), and the fecal DM produced daily (R, kg/d) were used to compute the digestible dry matter intake (D, kg/d), i.e., $D=F-R$. The digestible amount of a specific nutrient was denoted D_{Nut}, namely, D_{NFC}, D_{NDF}, D_{CF}, D_{Ash}, D_{NDS} or D_{CP}, and its concentration in the diet was denoted as $[D_{Nut}]$. For a given nutrient, the individual components of Eq. (12) were computed as follows: $Offered_{Nut}=10^{-6}\sum_j [DM]_j[Nut]_j x_j$; $O_{Nut}=10^{-6}[DM]_O[Nut]_O O$; and $R_{Nut}=0.001[Nut]_{feces}R$. The amount of a given nutrient in the fecal DM was also denoted R_{Nut} (kg/d). A given nutrient intake was calculated as $F_{Nut}=Offered_{Nut}-O_{Nut}$. The coefficients of digestibility of the diet DM and of a specific nutrient were determined as follows:

$$[D](\mathrm{g/kg}\ DM) = 1000D/F \quad (14)$$

$$[D_{Nut}](\mathrm{g/kg}\ DM) = 1000D_{Nut}/F \quad (15)$$

The daily urinary energy (UE, MJ/d) was estimated from the urinary nitrogen content with the equation described by Paladines et al. (1964), and the daily methane energy (E_g, MJ/d) was estimated with the equation proposed by Blaxter and Clapperton (1965)[1], modified as Eq. (16):

$$E_g = 18.8F(1.3+0.112[D]_m+L_c(2.37-0.05)[D]_m)/100 \quad (16)$$

The terms $[D]_m$ and L_c represent the mean digestibility of the maintenance level diet ($L=1$) and the corrected plane of nutrition, respectively. These waste energy estimates were discounted from DE to yield the apparent metabolizable energy intake (ME) of the diets ([ME]) fed to each animal at each treatment × period × animal combination:

$$ME(\mathrm{MJ/d}) = DE-UE-E_g \quad (17)$$

Nonetheless, the actual L_c value was unknown and an additional equation relating L_c to another independent input of the metabolizable energy intake was necessary to find a numerical solution for L_c. According to Eq. (6), $L_c=ME/M_m$; and $M_m=(FHP+A)/k_m$ and $k_m=0.35ME/(18.8F)+0.503$ (AFRC, 1993). The terms FHP, A, and k_m represent the fasting heat production, voluntary activities, and efficiency of utilization of the ME for maintenance, respectively. By rearranging terms, isolating ME, and taking only the positive root as the solution for ME, we have:

$$ME(\mathrm{MJ/d}) = (-0.503+(0.503^2-4\times(0.35/(18.8F))\times(-L_c(FHP+A)))^{1/2})/(2\times 0.35/(18.8F)) \quad (18)$$

The unknown corrected plane of nutrition (L_c) was solved by taking the output of the ratio Eq. (17)/Eq. (18) as the objective function and by numerically varying L_c using the optimization tool of Microsoft Excel Solver®. A solution was considered reached whenever the ratio Eq. (17)/Eq. (18) approached 1.0000 ± 0.0005. Consequently, the metabolizable energy content taken from the results of Eq. (17) (or Eq. (18)) based on the optimized L_c was finally obtained as $[ME]=ME/F$ (MJ/kg DM), and the corrected diet metabolizability computed as $q'_m=[ME]/18.8$. The nitrogen balance (NB, g/d) was computed as $NB=(F_{CP}-R_{CP})/6.25-UN$, in which UN (g/d) is the amount of urine nitrogen excreted on a daily basis.

2.5. Statistical analysis

The intake of nutrients, digestible nutrients, digestible energy, and the amounts of feces and urine produced daily were scaled to the metabolic body size, i.e., by dividing the respective intake or amount excreted by $W^{3/4}$ (g or MJ/(d× kg$^{3/4}$)). The exception was the amount of fiber intake that was scaled to W at its first power (g/(d × kg)) (Van Soest, 1994). The linear mixed model described by Eq. (19) was fitted to the weight-scaled variables after logarithmic transformation, and to the nutrient contents (g/kg DM) transformed as $2\arcsin\sqrt{0.001[Nut]}$. Nonetheless, the estimated variables were presented in its full scaled form, i.e., as g or MJ/(d × kg$^{3/4}$), g/(d × kg), or g/kg DM. The following linear mixed statistical model was adopted (Tempelman, 2004):

$$Y_{ikl} = \mu+\alpha_i+s_k+\beta_l+\alpha\beta_{il}+e_{ikl} \quad (19)$$

in which Y_{ikl} is the observation related to the variable measured in the k-th sheep fed to the i-th plane of nutrition during the l-th period. The fixed effects in Eq. (19) are the mean (μ), the plane of nutrition (α_i), the periods for the two simultaneous balanced Latin squares (β_l), and the treatment by period interaction ($\alpha\beta_{il}$). The random effects are sheep (s_k) and the usual error term (e_{ikl}). The statistical model was fitted using the PROC MIXED procedure of SAS (version 9; SAS Institute Inc., Cary, NC, USA) with restricted maximum likelihood (REML) as the estimation method. The repeated command was used with s_k as subjects. The variance-covariance matrix was modeled as variance components, compound symmetry, first order auto-regressive correlations, and as the unrestricted variance–covariance structure (Littell et al., 2006). The likelihood of the different variance–covariance structures was assessed from the Bayesian information criterion (BIC) proposed by Schwarz (1978). The BIC is a SAS output obtained after fitting Eq. (19) with different variance-covariance structures to the data (Wolfinger, 1993).

[1] Although calculations made by Blaxter and Clapperton (1965) were correct, their equation was printed incorrectly. It can be easily demonstrated that, on their terms, CH_4(kcal/100 kcal feed)$=1.30+0.112D+L(2.37-0.050D)$.

Provided that the likelihoods computed from both BIC or Akaike information criteria converge (Burnham and Anderson, 2004), the likelihoods of the different variance–covariance models were computed (Vieira et al., 2012). The computed information criteria (BIC, likelihood probability, and evidence ratio) were also used to check the likelihood of Eq. (19) fitted to data by introducing treatment grouping in the repeated sentence of the SAS program to check the homoscedasticity assumption (Wolfinger, 1996; Littell et al., 2006). Null hypotheses regarding treatment factors and their linear and quadratic effects were rejected for $P<0.05$.

For significant regressions, the predicted 95% confidence intervals (95% *CI*) at the different values of L were presented as follows: $\hat{y}_L \pm (\widehat{Ur}-\widehat{Lr})/2$; in which $\hat{y}_L$ is the predicted response at a given L; and $\widehat{Ur}$ and $\widehat{Lr}$ are the predicted upper and lower limits of the 95% *CI*, respectively. Given the absence of treatment effects for a given variable, the least squares estimates of the 95% *CI* for each L was provided as $\overline{y}_L \pm (\overline{Ur}-\overline{Lr})/2$, where $\overline{y}$ is the least squares mean, and $\overline{Ur}$ and $\overline{Lr}$ are the respective upper and lower least squares limits of the 95% *CI* for the mean.

Some variables were reanalyzed by regressing observed values against the corrected plane of nutrition (L_c from Eq. (17)/Eq. (18) ratio). Some variables exhibited curvilinear and asymptotic behaviors; other variables presented linear behaviors. However, these observed behaviors presented an initial phase near maintenance in which an average response was followed by the respective curvilinear or linear ascending behavior above maintenance. For this reason, we adopted two models to describe these two possible relationships, as shown in Eqs. (20)–(22):

$$\text{If } \min L_c \le L_c \le L' \text{ then } y=\overline{y}_0;\ \text{for } L_c>L' \text{ then } y=A-B\exp(-kL_c) \tag{20}$$

$$\text{If } \min L_c \le L_c \le L' \text{ then } y=\overline{y}_0;\ \text{for } L_c>L'\ y=\theta_0+\theta_1 L_c \tag{21}$$

$$\text{If } \min L_c \le L_c \le L' \text{ then } y=\overline{y}_0;\ \text{for } L_c>L' \text{then } y=\theta_1 L_c \tag{22}$$

The minimum (min) L_c was taken as the lowest observed L_c value within the entire L_c range. The parameter $\overline{y}_0$ was the mean of the observations that did not change within the maintenance range, which, in turn, is defined by its upper limit L'. In Eq. (20), parameter A is the asymptotic response for a given value of y, B is a scale parameter, and the rate of increase in y reduces asymptotically at a fractional rate k for $L_c>L'$. The intercept θ_0 can be excluded from Eq. (21) to yield the no-intercept model described in Eq. (22) as a possible alternative chosen on the basis of the likelihood of Eqs. (21) and (22). The regression coefficient (θ_1) corresponds to the unit increase in the dependent variable for each increasing unit in L_c above L'. The dependent variables (y) were scaled to W or $W^{3/4}$ accordingly, and Eqs. (20)–(22) were fitted to the scaled data by means of the robust iteratively reweighted nonlinear least squares of the PROC NLIN procedure (SAS, version 9). The sums of squares of the errors obtained after fitting Eqs. (20)–(22) were used to compute likelihood criteria, as suggested by Vieira et al. (2012). No inferences were drawn above the maximum or below the minimum observed L_c values.

3. Results

The fit of Eq. (19) to the variables listed in Table 2 using different variance–covariance structures revealed that variance components structure was the best choice among the tested variance-covariance structures for the variables W, ODM, O, F, F_{CP}, F_{NDF}, R, R_{CP}, R_{NDF}, R_{NFC}, R_{NDS}, $[UN]$, UN, DE, F_{OM}, and $[D_{OM}]$. The compound symmetry structure with constant correlations among repeated measurements taken on the same sheep across periods was the best choice for the variables F_{CF}, F_{Ash}, q'_m, and D_{OM}. The unstructured or unrestricted variance-covariance structure was the best choice for the variables D, F_{NFC}, F_{NDS}, R_{Ash}, R_{CF}, and L_c. The amounts of orts left by the animals presented heterogeneous variances among treatments.

The liveweight of the animals (W) was affected by the plane of nutrition (Tables 2 and 4). The predicted W values peaked for treatments between 1.5 × and 2 × maintenance.

Table 2
P-values related to the measured variables analyzed for the effects of the planned plane of nutrition, periods, and treatment by period interaction.

Variable[a]	*P*-values associated to the effects of the statistical model				
	Treatment	Period	Interaction	Linear	Quadratic
W[a], kg	<0.001	0.075	0.421	0.163	<0.001
ODM[b]	<0.001	0.069	0.402	<0.001	0.415
O[b]	0.057	0.210	0.762	0.011	0.046
F[b]	<0.001	0.150	0.444	<0.001	0.276
F_{OM}[b]	<0.001	0.156	0.436	<0.001	0.250
F_{CP}[b]	<0.001	0.057	0.446	<0.001	0.841
F_{CF}[b]	<0.001	0.104	0.561	<0.001	0.007
F_{Ash}[b]	<0.001	0.029	0.766	<0.001	0.758
F_{NDF}[b]	<0.001	0.083	0.419	<0.001	0.156
F_{NFC}[b]	0.001	0.135	0.382	<0.001	0.814
F_{NDS}[b]	0.002	0.097	0.393	<0.001	0.846
R[c]	<0.001	0.939	0.861	<0.001	0.002
R_{CP}[c]	<0.001	0.148	0.630	<0.001	0.139
R_{CF}[c]	0.001	0.348	0.212	0.001	0.006
R_{Ash}[c]	0.002	0.063	0.375	0.002	0.761
R_{NDF}[c]	<0.001	0.954	0.681	<0.001	0.013
R_{NFC}[c]	<0.001	0.095	0.670	<0.001	0.007
R_{NDS}[c]	<0.001	0.821	0.932	<0.001	0.005
$[UN]$[d]	0.031	0.759	0.232	0.012	0.375
UN[e]	0.023	0.531	0.280	0.028	0.334
D[b]	0.037	0.067	0.172	<0.001	0.024
DE[b]	0.001	0.035	0.113	<0.001	<0.001
D_{OM}[b]	<0.001	0.022	0.059	<0.001	0.365
$[D_{OM}]$[a]	0.040	0.835	0.357	0.680	0.004
q'_m[a]	0.025	0.313	0.454	0.020	0.039
L_c[a]	0.001	0.017	0.194	<0.001	0.099

[a] W is liveweight (kg), and the dimensionless variables are the corrected metabolizability (q'_m), the corrected plane of nutrition (L_c), and the coefficient of digestibility of the organic matter ($[D_{OM}]$).

[b] Amounts of offered dry matter (ODM) and orts (O), and intakes of dry matter (F), crude protein (F_{CP}), crude fat (F_{CF}), ash (F_{Ash}), neutral detergent fiber (F_{NDF}), non-fibrous carbohydrates (F_{NDF}), neutral detergent solubles (F_{NDS}), digestible dry matter (D), digestible energy (DE), organic matter (F_{OM}), and digestible organic matter (D_{OM}), all expressed as $g/(d \times kg^{3/4})$ or $MJ/\left(d \times kg^{3/4}\right)$.

[c] Amounts of fecal dry matter (R), crude protein (R_{CP}), crude fat (R_{CF}), ash (R_{Ash}), neutral detergent fiber (R_{NDF}), non fibrous carbohydrates (R_{NFC}), and neutral detergent solubles (R_{NDS}), all expressed as $g/(d \times kg^{3/4})$.

[d] Urinary nitrogen ($[UN]$, g/kg urine DM).

[e] UN, $g/(d \times kg^{3/4})$.

The least squares means for periods one, two, three, and four were 44.9, 42.6, 41.8, and 44.3 kg, respectively, with a common half 95% *CI* error equal to 1.7 kg.

All variables presented in Table 2 were affected by the planned plane of nutrition (L). The natural logarithm (log) of the actual or corrected plane of nutrition (L_c) increased linearly with L. Some variables were affected by periods and no variable was affected by the $\alpha\beta_{il}$ effect. There were linear and quadratic effects for the log transformed offered feed and orts, respectively. With the exception of the L^2 effect over $\log F_{CF}$, only linear effects of L were observed on the log intake rates of the other nutrients (F, F_{CP}, F_{Ash}, F_{NDF}, F_{NFC}, F_{NDS}, and F_{OM}). The predicted values of F and F_{OM} (and so forth for other nutrients) were not exactly the same as the difference between the least squares means computed for the offered and orts dry matter because F and F_{OM} were analyzed directly as dependent variables after transformations (Tables 3 and 4). With the exception of the linear effects observed for fecal crude protein (R_{CP}) and ash (R_{Ash}), the amounts of fecal dry matter (R) and of nutrients voided in feces (R_{CF}, R_{NDF}, R_{NFC}, *and* R_{NDS}) varied according to L^2 asymptotically (values not shown).

The plane of nutrition linearly affected both the urine nitrogen content ([*UN*], g/kg of urine *DM*) and the urine nitrogen excreted on a daily basis scaled to the metabolic body size (*UN*, $\text{g}/(\text{d} \times \text{kg}^{3/4})$), as presented in Tables 2 and 4.

No orts were left at the maintenance level but there was a weak evidence that orts increased as L increased (Tables 2 and 3). The orts at 1.5 × and 2 × maintenance amounted to 2.9 and 3.0% of the offered dry matter, respectively. The offered *DM* varied because it was scaled to $W^{3/4}$; however, the nutrient contents did not vary in a similar manner, which explains why we did not present error estimates for nutrient composition as a proportion of offered *DM*. The orts amounted to 8.1% of the offered *DM* at 2.5 × maintenance (Table 3). In addition, the [*DM*], [*CP*], [*CF*], [*Ash*], [*NDF*], and [*NFC*] contents of the orts were linearly affected by L (Table 2).

The most appropriate variance–covariance structure for the nutrient contents in the orts was variance components. The fecal [*DM*], [*NDF*], and [*NFC*] contents were not affected by the plane of nutrition ($P = 0.594$, 0.440, and 0.077, respectively). The fecal [*Ash*] content varied according to L^2 ($P = 0.029$). The exception was the more likely unrestricted variance-covariance structure identified for the [*CF*] content of the feces, but no significant L and L^2 effects were detected for this variable ($P = 0.198$ and 0.738, respectively).

The scaled intake of a specific nutrient can be approximately computed from Table 3 by taking the offered *DM*, the orts *DM*, and the nutrient concentrations in the offered and orts *DM*. For instance, the scaled *NFC* intake at $L = 2$ was $0.001 \times (427.4 \times 49.6 - 701.0 \times 1.5) = 20.1\ \text{g}/(\text{d} \times \text{kg}^{3/4})$; the full-scale estimate can be computed as the product $W^{3/4} \times NFC$ for the predicted weight at $L = 2$, namely $46.0^{3/4} \times 20.1 = 355.0$ g/d of non fibrous carbohydrates.

There was a weak evidence that [*D*] was affected by the treatment levels ($P = 0.056$). A variance components structure was the best choice for [*D*], whereas the unrestricted variance–covariance structure was the best choice for [D_{CP}]. The compound symmetry structure with a constant correlation was the best variance-covariance structure for [D_{CF}], and a quadratic effect (L^2) was observed ($P < 0.001$). The [D_{NDF}] and [D_{NFC}] were affected by L^2 ($P = 0.004$ and 0.029, respectively), and the variance components

Table 3
Amounts[a] of dry matter (*DM*) and nutrients[b] in the offered, orts, feces, and digested matter at each planned plane of nutrition (L) of the experimental diets fed to the $F1$ Santa Inês × Dorper wethers.

Plane of Nutrition	*DM*[c]	[*DM*][d]	[*CP*][d]	[*CF*][d]	[*Ash*][d]	[*NDF*][d]	[*NFC*][d]
Offered							
$L=1$	28.3 ± 0.9	374.1	171.9	54.9	63.0	350.2	364.3
$L=1.5$	37.5 ± 0.8	364.6	197.6	50.4	58.0	357.9	339.7
$L=2$	49.6 ± 1.0	364.1	127.4	48.7	65.8	334.0	427.4
$L=2.5$	65.6 ± 2.1	362.3	148.8	53.0	63.2	360.9	377.5
Orts							
$L=1$	–	–	–	–	–	–	–
$L=1.5$	1.1 ± 1.1	426.9 ± 3.7	109.4 ± 40.2	53.6 ± 17.7	66.5 ± 15.1	91.9 ± 26.0	674.8 ± 34.2
$L=2$	1.5 ± 1.5	432.9 ± 2.4	44.7 ± 18.9	32.9 ± 9.9	73.1 ± 11.2	145.5 ± 22.5	701.0 ± 23.7
$L=2.5$	5.3 ± 3.9	432.8 ± 2.0	46.0 ± 16.5	37.9 ± 9.1	77.3 ± 9.9	140.7 ± 19.2	694.7 ± 20.6
Feces							
$L=1$	6.5 ± 0.8	357.7 ± 6.4	154.7 ± 16.9	36.5 ± 7.7	163.7 ± 17.3	514.5 ± 24.2	125.0 ± 27.7
$L=1.5$	10.0 ± 0.9	359.3 ± 6.4	140.3 ± 16.2	39.4 ± 7.6	137.3 ± 9.3	513.2 ± 24.2	163.2 ± 30.9
$L=2$	12.5 ± 1.1	353.7 ± 6.4	130.2 ± 15.7	32.6 ± 5.8	129.9 ± 16.7	536.5 ± 24.1	170.8 ± 31.5
$L=2.5$	12.9 ± 1.5	359.3 ± 6.4	154.7 ± 16.9	30.4 ± 4.9	140.2 ± 26.6	515.4 ± 24.2	152.6 ± 30.1
Digested							
$L=1$	21.9 ± 1.4	764.3 ± 27.4	135.5 ± 4.2	46.1 ± 1.9	23.4 ± 2.5	235.8 ± 22.6	330.2 ± 10.6
$L=1.5$	26.7 ± 1.8	735.7 ± 28.5	163.7 ± 2.7	39.8 ± 1.5	20.3 ± 4.5	207.8 ± 16.4	280.8 ± 10.2
$L=2$	34.7 ± 2.8	731.0 ± 28.6	95.5 ± 3.9	40.2 ± 1.5	30.9 ± 3.9	215.6 ± 16.6	367.9 ± 10.9
$L=2.5$	47.8 ± 3.6	783.1 ± 26.6	124.8 ± 10.1	47.1 ± 1.9	31.2 ± 6.3	260.5 ± 23.3	308.3 ± 10.2

[a] Estimates without errors are analytical results about the offered *DM*. Other estimates are 95% *CI* for each L.
[b] *CF*, crude fat; *CP*, crude protein; *NFC*, non fibrous carbohydrates; *NDF*, neutral detergent fiber assayed with amylase and Na_2SO_3, and expressed exclusive of residual ash.
[c] $\text{g}/(\text{d} \times \text{kg}^{3/4})$.
[d] g/kg of *DM* for the offered, orts, feces, and digested materials.

Table 4
Confidence intervals (95% *CI*) for some measured variables predicted at each plane of nutrition as planned (L).

Variable	95% *CI* predicted at each plane of nutrition as planned			
	$L=1.0$	$L=1.5$	$L=2.0$	$L=2.5$
W[a]	40.1 ± 2.0	45.3 ± 1.6	46.0 ± 1.6	52.1 ± 2.0
F[b]	28.4 ± 1.2	36.6 ± 1.0	47.2 ± 1.3	60.9 ± 2.6
F_{OM}[b]	26.6 ± 1.1	34.3 ± 1.0	44.2 ± 1.2	57.0 ± 2.4
$[UN]$[c]	90.3 ± 19.3	104.1 ± 13.3	118.7 ± 14.1	134.2 ± 22.6
UN[d]	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.2
NB[d, e]	0.1 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2
q'_m[a]	0.619 ± 0.042	0.601 ± 0.037	0.620 ± 0.037	0.677 ± 0.041
L_c[a]	0.93 ± 0.12	1.28 ± 0.13	1.77 ± 0.15	2.45 ± 0.24

[a] W is liveweight (kg), and the dimensionless variables are the corrected metabolizability (q'_m) and the corrected plane of nutrition (L_c).
[b] Intakes of dry matter (F) and organic matter (F_{OM}), both expressed as g/(d × kg$^{3/4}$).
[c] Urinary nitrogen ([UN], g/kg urine DM).
[d] UN, g/(d × kg$^{3/4}$).
[e] NB, nitrogen balance expressed as g/(d × kg$^{3/4}$).

structure was the most suitable structure for these two variables. The unstructured variance–covariance matrix was best fitted to [D_{CP}] and [D_{Ash}]; however, no significant treatment effects were detected for either variable. The intake rate of a digestible nutrient can be approximately computed from Table 3 by taking the product of nutrient digestibility (g/kg DM) and the respective intake of digested dry matter; e.g., for D_{CP} at $L=2$, we obtain a value of $34.7\times0.001\times95.5=3.3$ g/(d × kg$^{3/4}$). The full scale intake for $L=2$ was calculated as $46.0^{3/4}\times3.3=58.3$ g/d of digestible crude protein.

The value of q'_m was affected by L^2, whereas L_c was affected only linearly by L (Tables 2 and 4). It is important to remember that these two variables were transformed as 2arc sin $\sqrt{q'_m}$ and log (L_c) prior to being fitted by Eq. (17), and the final expressions for the full scale q'_m and L_c were the respective squared senoid and exponential functions as depicted in panels (a) and (b) of Fig. 1, respectively. The planned metabolizability ($q_m=0.55$, Eq. (4)) was below the predicted lower bound of the 95% *CI* of q'_m at all planned levels of intake (Table 4). The corrected metabolizability could be considered nearly constant due to the predicted interval estimates at $L=$ 1, 1.5, and 2 × maintenance; nonetheless, at $L=2.5$, the animals were able to select the richest diet, as shown by the amounts of orts left behind (Fig. 1a).

The full scale L_c was curvilinear, as depicted in panel (b) of Fig. 1 and the predicted 95% *CI* estimates at each L level are shown in Table 4. Therefore, the planned L was within the 95% *CI* estimates for L_c only for $L=1$ and 2.5; the actual or corrected plane of nutrition (L_c) was below the planned values at 1.5 × and 2 × maintenance.

The intakes of dry matter (F, Fig. 1c and Table 4), digestible dry matter (D, Fig. 1c), and organic matter (D_{OM}, Fig. 1d) increased in an asymptotic fashion as L_c increased. Below and near maintenance (≅ 1 ×), however, observed values were constant. The Eq. (19) used to describe these variables are mostly descriptive, and the extrapolation of inferences for $L_c<0.8$ and $L_c>2.9$ is not recommended.

The intakes of digestible organic matter (D_{om}, Fig. 1d), digestible energy (DE, Fig. 1e), and neutral detergent fiber (F_{NDF}, Figure 1 f) increased linearly above maintenance. This means that the linear segmented model presented a greater likelihood than Eq. (19) when describing these three variables. Eq. (20) was necessarily used to describe the observed behaviors because intakes of these nutrients at a common threshold below maintenance ($L_c<0.9$) were constant.

The fit of Eqs. (19)–(20) to the data revealed common tendencies among variables, as depicted in both Figs. 1 and 2. Nonlinear asymptotic behaviors were observed for the scaled intakes of F_{NDS}, digestible total carbohydrates (D_{TC}), F_{Ash}, and D_{Ash} (panels (a), (b), and (c) of Fig. 2), whereas a crescent linear behavior was observed for F_{CP}, D_{CP}, F_{CF}, and D_{CF} (panels (a), (b), and (c) of Fig. 2).

The difference between the nitrogen intake and the nitrogen excreted in both feces and urine, i.e., the nitrogen balance (NB, Table 4) expressed on a daily basis as g/(d × kg$^{3/4}$), was not affected by the plane of nutrition ($P=0.429$), experimental periods ($P=0.194$), or the treatment × period interaction ($P=0.871$). Although some correlation among repeated measures taken at different periods on the same sheep (compound symmetry) might have occurred, the best choice for the covariance structure was variance components because of its simplicity and comparable likelihood to that of the compound symmetry structure.

4. Discussion

The traditional Latin square design has been analyzed assuming the nonexistence of the $\alpha\beta_{il}$ term and other possible interactions (Littell et al., 2006; Lucas, 1957; Neter and Wasserman, 1974). Although included in Eq. (17) as recommended by Tempelman (2004), the effect of the interaction $\alpha\beta_{il}$ was not significant for any dependent variables studied (Table 2). In addition, the investigation of the possible variance-covariance structures (Littell et al., 2006) based on likelihood calculations (see section 2.5) revealed that errors were correlated in some cases, i.e., covariances or correlations among measurements taken on the same sheep across periods were likely to have occurred for some variables measured in the present

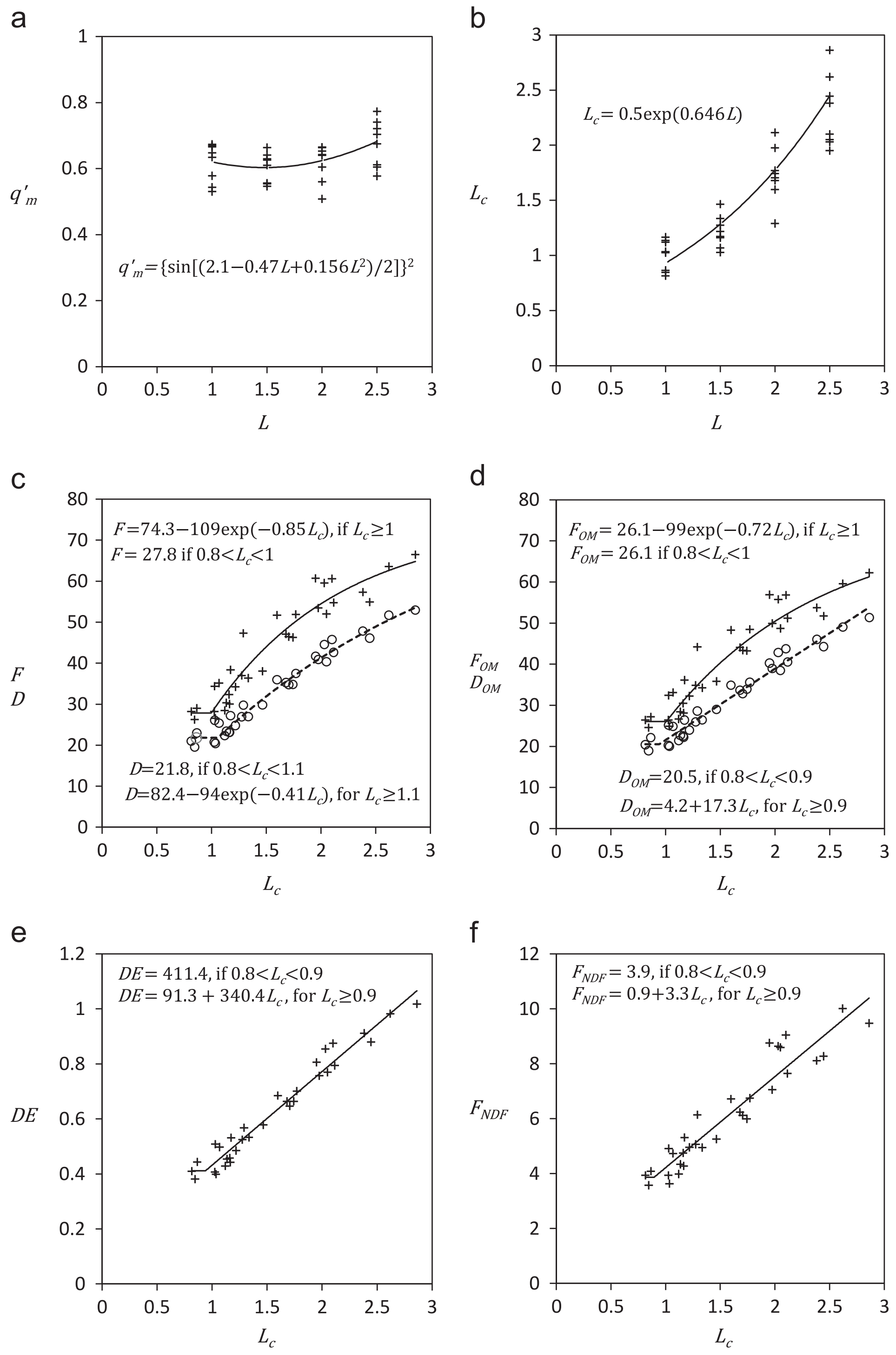

Fig. 1. Trends observed for some variables as functions of the planned plane of nutrition (L, dimensionless) and the corrected plane of nutrition (L_c, dimensionless). On panel (a) the corrected observed (+) and predicted (solid line) metabolizabilities (q'_m, dimensionless) are plotted against L. On panel (b) observed (+) and predicted (solid line) L_c values are plotted against L. On panel (c) are plotted the observed dry matter intake g/(d x kg$^{3/4}$), +), the predicted F (solid line), the observed digestible dry matter intake (D, o), and the predicted D (dashed line). On panel (d) are shown the organic matter (F_{OM}, +) and digestible organic matter (D_{OM}, g/(d × kg$^{3/4}$), o) intakes, as well as the predicted values for F_{OM} (solid line) and D_{OM} (dashed line). On panel (e) are depicted the observed (+) and predicted (solid line) digestible energy intake (0.001 × DE, MJ/(d × kg$^{3/4}$)). On panel (f) are depicted the observed (F_{NDF}, g/(d × kg), +) and predicted (solid line) insoluble fiber intake.

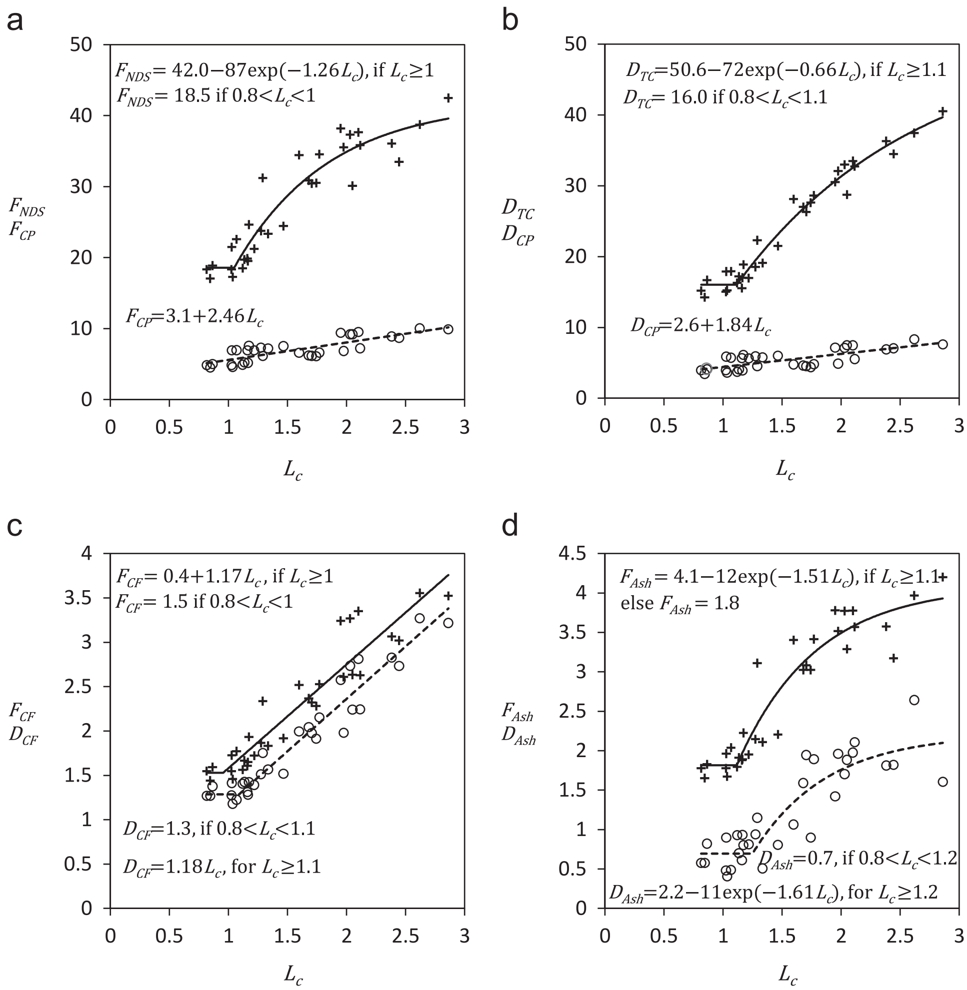

Fig. 2. Trends observed for some variables as functions of the corrected plane of nutrition (L_c, dimensionless). On panel (a) the observed (+) and predicted (solid line) neutral detergent solubles (F_{NDS}, g/(d × kg$^{3/4}$)) intakes, and the observed (o) and predicted (dashed line) crude protein intakes (F_{CP}, g/(d × kg$^{3/4}$)) are plotted against L_c. On panel (b) observed (+) and predicted (solid line) total of digestible carbohydrates intakes (D_{TC}, g/(d × kg$^{3/4}$)), and observed (o) and predicted (dashed line) digestible crude protein intakes (D_{CP}, g/(d × kg$^{3/4}$)) are plotted against L_c. On panel (c) are plotted the observed intake of crude fat (F_{CP}, g/(d × kg$^{3/4}$), +) and its predicted value (solid line), and the observed digestible crude fat intake (D_{CF}, g/(d × kg$^{3/4}$), o) and its predicted value (dashed line). On panel (d) are shown the observed (+) and predicted (solid line) F_{Ash} intakes expressed as (F_{Ash}, g/(d × kg$^{3/4}$)), and the observed (D_{Ash}, g/(d × kg$^{3/4}$)) and predicted (dashed line) digestible ash intakes.

study, thus indicating that the assumption of independent or uncorrelated errors for the simultaneous Latin square design does not always hold true.

The field of operations research deals with the problem of allocating scarce resources; it is from this field that the idea of diet optimization arose. The most common objective function used is the cost of the diet, as shown in Eq. (1), which the model aims to minimize (Bazaraa and Shetty, 1979). The problem of feeding a particular type of farm animal occurs in situations where resources are scarce. Therefore, the controlled feeding system in which the animal is expected to eat all of an offered diet is of interest (Emmans and Kyriazakis, 1995); in our case, we aimed to produce an optimized diet at minimal cost. The existence of slightly increasing amounts of orts indicated that too much feed was offered only at the highest plane of nutrition (Table 3); however, the definition of a controlled feeding situation was probably still applicable. Therefore, if the objective of the research is to guarantee the intake of the entire amount of the offered diet to minimize systematic variations in metabolizability of the diet effectively consumed, the researcher should be aware that, at high levels of feeding, the animals might be capable of selecting the most nutritious part of the diet (Fig. 1a); therefore, to assure a controlled feeding system, no orts should be allowed.

A good estimate of the voluntary feed intake of the animal and estimates of the metabolizable energy contents for maintenance and production of the selected feedstuffs are required for linear programming (AFRC, 1993). Therefore, for the linear programming of diets for ruminants in the current feeding systems, voluntary dry matter intake must be input to produce the expected output of animal performance (McDonald et al., 1995; Fox et al., 2004). The use of iterative linear programming may overcome this problem and provides a tool for the ration formulator, but the program is not simple to implement and demands professionally programmed software (Munford, 1996). In contrast, with nonlinear programming, intake could be treated as an output and computed on the basis of the unknowns x_j as $\sum_j 0.001[DM]_j x_j$. In addition, performance (*e.g.*, ΔW) can be constrained to a target or desired performance level and treated as an input or an additional constraint. In the present work, however, ΔW was unconstrained in Eq. (3) because we were interested in intake and digestibility behaviors; therefore, no inferences were made regarding ΔW because short experimental periods may preclude valid inferences about liveweight gain (Eaton et al., 1959). A rather complete evaluation of the model could be accomplished if observed performances are compared to the expected ones (Joyce et al., 1975; Henrique et al., 2005; 2011; Tedeschi et al., 2010).

It has long been recognized that the nutritive value of a unit weight of feed is not constant and depends on the amount of feed consumed, as the greater the feed intake, the lower the feed utilization (Blaxter, 1956; Brody, 1945; Tedeschi et al., 2010; Van Soest and Fox, 1992). For the same reason, discounts for net energy and protein must be applied for a given diet fed at multiples of maintenance levels (Van Soest and Fox, 1992); similarly, with increasing nutritional levels in the diet, the metabolizability (q_m) of the diet is expected to decline (Blaxter, 1966). For this reason, we tried to keep the metabolizability of the dietary energy and the $MP:ME$ ratio constant (Table 2) to verify the ability of the optimization tool to provide predictable amounts of nutrients and digestible nutrient intakes according to the current theories underlying the feeding of ruminants (Conrad et al., 1964; Mertens, 1987; AFRC, 1993; NRC, 2007; Allen et al., 2009) using sheep as a generalizing model (Fig. 1). The nonlinear constraints used (Eqs. (6)–(8)) are more natural because the true energy and protein values of a diet are essentially nonlinear, given the dependence of the nutritive value on the amount of feed eaten. The main advantage of the nonlinear optimization is the freedom it provides and the flexible way in which constraints can be built. There is no need for linearization or transformations of any kind, and constraints can be programmed as originally conceived. In addition, the user can program a specific model in the spreadsheet by pooling parts of different models that are more accurate in predicting specific variables of interest, as suggested by Offner and Sauvant (2004), so that the overall performance of the new model may be improved.

We did not check the protein metabolizability of the diets, but the majority of the protein losses occurred as fecal protein (R_{CP}, Table 2). The amount of R_{CP} ($\mathrm{g/(d \times kg^{3/4})}$) can be easily computed from Table 3 as $R \times fecal\,[CP]$. We expected that urinary energy excretion would be in close agreement with crude protein intake (Brody, 1945), as urinary energy and nitrogen contents are highly correlated (Street et al., 1964). However, there was only a slightly significant increase in the daily urinary nitrogen excretion (section 3), i.e., $0.1\ \mathrm{g/(d \times kg^{3/4})}$, per unit increase in L. In addition, as mentioned previously, all fecal losses appeared to increase in an asymptotic fashion (not shown), but the nitrogen balance was nearly constant in the L range studied. The constant $MP:ME$ ratio adopted might have favored the retention of protein and reduced the nitrogen and energy losses (Blaxter, 1966; Fox et al., 2004). As a result, animals became heavier with different diets. The changes in liveweight that occurred with L^2 are difficult to explain (Table 2). Growth measures are less variable with longer experimental periods (Eaton et al., 1959), which might explain the absence of the period effect. Therefore, as was the case with growth, measures related to protein and energy retention demand continuous rather than change-over trials to verify the $MP:ME$ ratio hypothesis.

The q'_m values were used to compute L_c values (section 2.4; Figs. 1a and 1b, and Table 4). Apparently, the animals were capable of performing nonlinear adjustments within the studied range of the planned plane of nutrition (L). Blaxter (1956; 1966) and Van Soest (1994) argued that variations in energy utilization of concentrates are lower than variations in the efficiencies of utilization observed for forages. In addition to the effect of dietary selection on q'_m at the highest level of L, the observed systematic difference between q_m (Eq. (4)) and q'_m (Fig. 1a) could be attributed to the tabular $[ME]$ value of the corn silage adopted (Table 1). One possible explanation for this was the use of a grain-producing variety with a shorter plant height instead of a silage-specific variety. The possibly greater proportion of corn grains (not quantified) might have resulted in a greater $[ME]$ value than the tabular value attributed to the corn silage used in the present study.

The intake and digestibility behaviors observed were highly predictable given that the patterns predicted by the models mimicked those exhibited by the observed data (Figs. 1 and 2). The curvilinear asymptotic responses of F, F_{OM}, F_{NDS}, and D_{TC} were in agreement with current theories related to the regulation of intake (Conrad et al., 1964; Mertens, 1987; Allen, 1996; Allen et al., 2009). The chemostatic nature of the regulation of intake appeared to operate as predicted, as intakes of D_{OM}, D_{CF}, DE, and F_{NDF} increased linearly in the L_c range (Figs. 1d, 1e, 1 f, and 2c). The value of F_{NDF} was constrained to a maximum intake of $12\ \mathrm{g/(d \times kg)}$ (Mertens, 1987; Vieira et al., 2008), though this limit was not reached within the observed L_c range. According to the biphasic theory of intake regulation (Conrad et al., 1964; Mertens, 1987; Allen, 1996), a plateau would be expected for L_c values greater than 2.5; however, this plateau was not reached. In this context, the segmented linear model has an inherent limited inference, because, otherwise, extrapolation would lead to the absurd situation in which the intake of a digestible nutrient would be greater than the intake of the nutrient itself (see Fig. 1d). Nevertheless, the models used (Eqs. (20)–(22)) provided a good representation of the behavior observed for nutrient and digestible nutrient intakes.

Depending on parameter estimates for the asymptotic behaviors of the intake of a nutrient and of the nutrient in the feces, a linear increase in the intake of the digestible nutrient is plausible for a given L_c range, as observed in panels (d) and (e) of Fig. 1 and in panels (b) and (c) of Fig. 2. Nonetheless, this model was only applied to demonstrate the theoretical behavior of the variables within the L_c range studied. Koong et al. (1982) defined "feeding level" as the ratio between gross energy intake and fasting heat production, and, to build their model based on nitrogen/energy metabolism of cattle, these authors postulated that the digestibility or absorbability of the feed protein or fat increases linearly as the "feeding level" increases. For carbohydrates, depending on parameter estimates of the model proposed by Koong et al. (1982), a curvilinear trend provides a possible solution and might mimic the principle of diminishing returns that is supported in Eq. (20). To some extent, Koong et al. (1982) anticipated the intake behavior of digestible nutrients observed in our study, that is, that the unit increase in nutrient intake and in the digestible nutrient intake per unit increase in the plane of nutrition ($L = ME/M_m$) were held constant within the observed L_c range (Figs. 1d, 1e, 2a, 2b, and 2c).

An interesting adjustment performed by the animals was observed in the form of a significant linear increase (Table 2) in F_{CP} and D_{CP} (Figs. 2a and 2b) despite the different [CP] levels in the offered diets. Pittroff and Kothman (1999) have argued that protein might be the driving force behind animals' feed-seeking behavior. Indeed, several metabolites and hormones are candidate signals molecules that regulate intake (Forbes, 2007); as a result, the biphasic theory of intake regulation (Conrad et al., 1964) has been put into question (Pittroff and Kothmann, 1999; Pittroff and Soca, 2006). Because ruminants can be raised in facilities or on pastures (or both), important differences in the feeding behavior can occur due to the feed offered, thus limiting inferences from intake predictions based on the biphasic theory of intake regulation (Pittroff and Soca, 2006). The calculated $MP:ME$ constraint, which was maintained approximately constant across the offered diets, allowed predictable responses of nutrient and digestible nutrient intakes for the controlled feeding situation performed in the present study. The use of the nonlinear optimization tool to balance the offered diets based on the simple AFRC (1993) model appeared to work adequately for a controlled feeding situation in practice, as intake and digestibility of major nutrients behave in accordance with the chemostatic regulation of intake (Conrad et al., 1964; Mertens, 1987). This theory was recently revisited and refined as the hepatic oxidation theory of intake regulation (Allen et al., 2009). However, the possibility of any circumstantial effects of MP and ME on intake regulation should not be disregarded.

5. Conclusions

The intake and digestibility of major nutrients providing 1.0 to 2.5 × the metabolizable energy required at maintenance in wethers behave in accordance with current theories of intake regulation when the metabolizability of the dietary energy and the metabolizable protein to metabolizable energy ratio are held constant. This problem can be solved as a nonlinearly constrained optimization problem of minimum cost formulation of diets for ruminants. The major advantage of the spreadsheet optimization is the possibility of pooling the most accurate information gathered from different nutrition models that otherwise is not possible to obtain with professionally programed software.

Despite the possible bias in the mean energy metabolizability of the diets computed from tabular [ME] values in the present study, the corrected metabolizability of the dietary energy and the corrected plane of nutrition behaved as planned. Therefore, the AFRC (1993) model appears to yield both increasing curvilinear asymptotic and increasing linear responses related to nutrient and digestible nutrient intakes by sheep under a controlled feeding situation.

Conflict of interest

There is no conflict of interest.

Acknowledgments

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